In Vivo Efficacy of Humanized Ceftaroline Fosamil-Avibactam Exposures in a Poly microbial Infection Model

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Although Gram-positive cocci are the most common pathogens in diabetic foot infections, these infections often are polymicrobial. The objective of this study was to assess the efficacy of a simulated human dose of 600 mg ceftaroline fosamil–600 mg avibactam every 8 h as a 1-h infusion in a polymicrobial in vivo murine model. Seven isolates were used (3 methicillin-resistant Staphylococcus aureus [MRSA] isolates, 1 methicillin-susceptible S. aureus [MSSA] isolate, 1 Escherichia coli isolate, 1 Enterobacter cloacae isolate, and 1 Bacteroides fragilis isolate) in various combinations in an immunocompromised polymicrobial tissue infection to assess the efficacy of the simulated regimen. Each infection was comprised of at least one S. aureus isolate with a MIC of 0.25 to 1 µg/ml and one Enterobacteriaceae isolate with a MIC of 1 or 4 µg/ml. Eight of 16 infections also included B. fragilis, with a MIC of 0.5 µg/ml, as a third organism. Efficacy was evaluated after 24 h as the change in log_{10} CFU from the level of 0-h controls. Efficacy was seen against all isolate combinations, with at least a 1-log kill against Enterobacteriaceae and a minimum of a 2-log kill against S. aureus and B. fragilis isolates. These bacterial reductions correlate with free drug concentration above the MIC (ft > MIC) produced by the humanized regimen of 100, 86, and 56% at MICs of 1, 2, and 4 µg/ml, respectively. The humanized regimen of 600 mg ceftaroline fosamil–600 mg avibactam every 8 h as a 1-h infusion showed predictable efficacy against all infections tested in this model. These data support further clinical investigation of ceftaroline fosamil-avibactam for the treatment of polymicrobial tissue infections.

While the majority of skin and soft-tissue infections are caused by Staphylococcus aureus, these infections can be polymicrobial, involving Gram-positive and Gram-negative pathogens (1, 2). Polymicrobial infections are more common in patients with compromised vasculature, such as diabetics. While Gram-positive organisms are often present in newer wounds, such as cellulitis, Gram-negative and anaerobic organisms are often found in patients with chronic wounds that have been previously treated (3). Anaerobic organisms, such as Bacteroides fragilis, are rarely a sole pathogen and are most frequently found in ischemic or necrotic wounds. Failure to initiate appropriate therapy can lead to poor outcomes and the emergence of resistant pathogens, making these infections increasingly difficult to treat. Therefore, empirical therapy for these patients often involves multiple antimicrobials, such as vancomycin, for Gram-positive coverage and a broad-spectrum cephalosporin, such as cefepime, or an extended-spectrum penicillin, such as piperacillin-tazobactam, for Gram-negative coverage.

Ceftaroline fosamil, a broad-spectrum antimicrobial indicated for the treatment of complicated skin and skin structure infections, has potent in vitro activity against many Gram-positive and Gram-positive pathogens, including methicillin-resistant S. aureus (MRSA) and some Enterobacteriaceae isolates (4). While ceftaroline has extensive activity against common Gram-negative organisms, similar to other cephalosporins, it has limited activity against extended-spectrum beta-lactamases (ESBLs) and anaerobes (5). Avibactam, a non-beta-lactam beta-lactamase inhibitor, has the ability to extend the spectrum of activity of ceftaroline, thereby making the combination of ceftaroline fosamil-avibactam active against many ESBL-producing organisms (6, 7). The addition of avibactam also improves the in vitro potency of ceftaroline to some anaerobic organisms, such as B. fragilis and Prevotella (8). Here, we describe the activity of a simulated human dose of 600 mg ceftaroline fosamil–600 mg avibactam every 8 h as a 1-h infusion in a polymicrobial thigh infection model.

MATERIALS AND METHODS

Antimicrobial test agents. Commercially available ceftaroline fosamil (lot 00017D16 [potency, 100%]) was obtained from the Hartford Hospital Pharmacy Department, and analytical-grade avibactam (lot AFCH005151 [potency, 99.7%]; Forest Laboratories, Inc., New York, NY) was used for all in vivo analyses. Clinical vials of ceftaroline fosamil were reconstituted as described in the prescribing information and diluted as appropriate to achieve the desired concentrations; analytical avibactam powder was weighed in a quantity sufficient to achieve the required concentrations and reconstituted immediately prior to each in vivo experiment. The combined ceftaroline and avibactam solutions were stored under refrigeration and discarded within 24 h of reconstitution.

Bacterial isolates. Enterobacteriaceae and Staphylococcus aureus isolates were stored frozen at −80°C in double-strength skim milk (Remel, Lenexa, KS) and then subcultured twice onto Trypticase soy agar with 5% sheep blood (Becton, Dickinson, and Co., Sparks, MD) and grown for 18 to 24 h at 37°C prior to use in the experiments. The Bacteroides fragilis isolate was also stored in the same manner, except for being subcultured twice onto anaerobic Trypticase soy agar with 5% sheep blood (Becton, Dickinson, and Co., Sparks, MD) and grown for approximately 48 h under anaerobic conditions at 37°C prior to use in the experiments. Ceftaroline-avibactam MICs were determined by broth microdilution with a fixed concentration of 4 µg/ml avibactam according to CLSI guidelines.
Inoculum volume testing. Inoculum volumes of 0.1, 0.2, and 0.3 ml were tested in vivo to determine which inoculum provided adequate initial CFU of B. fragilis, S. aureus, and Enterobacteriaceae isolates and to assess tolerability to intramuscular injections of up to 0.3 ml in the animals. Each inoculum was made and diluted so that there was a total 10^7 CFU of each infecting organism.

Neutropenic thigh infection model. Pathogen-free, female ICR mice weighing approximately 20 to 22 g were acquired from Harlan Sprague Dawley, Inc. (Indianapolis, IN), and utilized throughout these experiments. Animals were maintained and used in accordance with National Research Council recommendations and provided food and water ad libitum. Mice were rendered neutropenic with 100 and 150 mg/kg of body weight intraperitoneal injections of cyclophosphamide (Cytoxan; Bristol-Myers Squibb, Princeton, NJ) given 1 and 4 days prior to inoculation, respectively. Three days prior to inoculation, mice were also given a single 5 mg/kg intraperitoneal injection of uranyl nitrate. This produces a predictable degree of renal impairment to slow drug clearance (9). Bacterial colonies of the Enterobacteriaceae and S. aureus strains were subcultured twice onto agar plates of Trypticase soy agar and 5% sheep blood (BAP) within 48 h before preparation of the inoculum. Bacterial colonies of the B. fragilis isolate were subcultured twice onto agar plates of anaerobic Trypticase soy agar and 5% sheep blood (BAP) within 48 h before preparation of the inoculum. Two hours prior to the initiation of antimicrobial therapy, each thigh was inoculated intramuscularly with a 0.1-ml solution containing approximately 10^7 CFU of each of the test isolates.

Determination of dosing regimen. A previously developed humanized dosing regimen of ceftaroline fosamil-avibactam was used (10). The free drug concentration above the MIC (f>T>MIC) exposure profile was similar between human and mouse, as noted in Table 1. In vivo efficacy. A total of 7 isolates were used (3 MRSA isolates, 1 methicillin-susceptible S. aureus [MSSA] isolate, 1 Escherichia coli isolate, 1 Enterobacter cloacae isolate, and 1 Bacteroides fragilis isolate) in various combinations in an immunocompromised polymicrobial thigh infection model against a simulated human dose of ceftaroline fosamil-avibactam. Each infection was comprised of at least one S. aureus isolate with a MIC of 0.25 to 1 μg/ml and one Enterobacteriaceae isolate with a MIC of 1 or 4 μg/ml. Eight of the 16 infections also included B. fragilis as a third organism. Beginning 2 h after inoculation, groups of three mice were administered the simulated human dose regimen of ceftaroline fosamil and avibactam over a 24-h period. All doses were administered as 0.2-ml subcutaneous injections. Control animals were administered normal saline with the same volume, route, and frequency as the treatment regimen. Groups of three untreated control mice were euthanized by CO2 exposure, followed by cervical dislocation just prior to the initiation of therapy (0 h). All other treatment and control mice were sacrificed 24 h after the initiation of therapy. Mice that did not survive to 24 h were harvested at the time of expiration. Following sacrifice, thighs were removed and homogenized individually in 5 ml of normal saline. Serial dilutions of the thigh homogenates were plated on agar plates of Bacteroides bile esculin agar to identify B. fragilis, Columbia CNA agar with 5% sheep blood to identify S. aureus, and MacConkey II agar to identify Enterobacteriaceae (Becton, Dickinson, and Co., Sparks, MD) for bacterial density determinations. MacConkey and CNA culture plates were incubated at approximately 37°C for 18 to 24 h in an ambient air atmosphere for bacterial growth. Bile esculin agar culture plates were incubated at approximately 37°C for 36 to 48 h in an anaerobic atmosphere. Efficacy, defined as the change in bacterial density, was calculated as the change between log_{10} CFU obtained for ceftaroline-avibactam-treated mice after 24 h and that for the 0-h control animals.

RESULTS

Bacterial isolates. The phenotypic profiles of all isolates used in the study are listed in Table 2. The presence of avibactam did not have a significant effect on S. aureus MICs; however, it did result in a minimum of a 3-dilution decrease in MICs for the Enterobacteriaceae and B. fragilis isolates.

Inoculum volume testing. The intramuscular injection of the inoculum up to a volume of 0.3 ml was well tolerated by the mice, with no notable differences between groups. The initial bacterial counts recovered for each organism from each inoculum size were similar. Since there was no difference between initial CFU recovered using various inoculum volumes, a volume of 0.1 ml was utilized, as this is the standard volume used for infections with one organism.

In vivo efficacy. The mean (± standard deviations [SD]) bacterial density for the 0-h control mice across studies was 6.68 ± 0.15, 6.20 ± 0.55, and 5.87 ± 0.16 log_{10} CFU for the Enterobacteriaceae, S. aureus, and B. fragilis isolates, respectively. The mean bacterial density of the 24-h control mice increased to 9.36 ± 0.50, 7.40 ± 0.57, and 7.67 ± 0.54 log_{10} CFU for the Enterobacteriaceae, S. aureus, and B. fragilis isolates, respectively. There were no observed inhibitory effects on the growth of individual isolates when tested in various combinations. Growth controls at 24 h for each isolate are shown in Fig. 1. Efficacy was seen against all combinations with at least a 1-log kill against Enterobacteriaceae isolates and a minimum of a 2-log kill against S. aureus and B. fragilis isolates (Fig. 2). Overall mean bacterial reductions (log_{10} CFU) for the E. coli (MIC = 1 μg/ml) and E. cloacae (MIC = 4 μg/ml) isolates were −1.55 and −1.16, respectively. Bacterial reductions for MRSA isolates with MICs of 0.25, 0.5, and 1 μg/ml were −2.71, −2.34, and −2.06, respectively. Similar bacterial reductions of −2.65 were noted for the MSSA isolate with a MIC of 1 μg/ml. Additionally, mean bacterial reductions of −2.58 were observed against the B. fragilis isolate.

DISCUSSION

Polymicrobial infections, such as complicated skin and skin structure and intra-abdominal infections, are responsible for substantial increases in morbidity and lead to a rise in health care expen-
ditures (11, 12). These infections involve pathogens that require the use of multiple antimicrobials, often complicating patient care and increasing the potential for drug-related adverse effects. While previous polymicrobial in vivo murine models have been conducted, these studies most often consisted of two organisms, typically B. fragilis and an Enterobacteriaceae isolate. This is the first study to our knowledge to incorporate up to three organisms in a single infection in a murine thigh model. Understanding the efficacy of antimicrobial agents against an infection comprised of multiple pathogens can provide great clinical utility, as these are often the infections seen in patients. In this study, a humanized regimen of 600 mg ceftaroline fosamil–600 mg avibactam every 8 h was evaluated against various polymicrobial infections in a neutropenic thigh infection model.

Efficacy was observed against all combinations of organisms tested in this model. Based on the pharmacodynamic profile of the simulated regimen and the MIC distribution of the isolates used, the activity of ceftaroline fosamil-avibactam observed is consistent with previous murine models. Similar bacterial reductions against the same S. aureus isolates used in this study were also observed in a previous murine thigh infection model conducted by our group using a simulated human dose of 600 mg ceftaroline fosamil every 12 h (13). Both regimens provided fT>MIC values above the known pharmacodynamic target of 30 to 40% that is required for bacterial killing of S. aureus; therefore, the efficacy observed is expected (14). Reductions of 1 to 2 logs in bacterial density were seen against both ESBL-producing Enterobacteriaceae isolates utilized, with MICs of 1 and 4 μg/ml where fT>MIC.
is 100 and 55%, respectively. When these Enterobacteriaceae isolates were tested as a single-organism infection against the same simulated human dose of ceftaroline fosamil-avibactam, similar bacterial reductions were observed (15). Additionally, the simulated regimen was effective against B. fragilis, with a greater than 2-log kill observed for each infection.

As noted above, based on the pharmacodynamic profile of the simulated human dose and the targets required, the efficacy observed in this model is not surprising. However, it is important to note that the total bacterial load of each infection is substantially greater than those of most other infections used in murine models, as each inoculum consisted of up to three organisms with 10⁷ CFU of each bacterium. Furthermore, a synergistic relationship between B. fragilis and Gram-negative aerobic organisms, such as Enterobacteriaceae, has been previously described which has been shown to increase mortality and overall bacterial growth (16, 17). This relationship and the rapid increase in bacterial growth can make it more difficult to treat these infections than to treat single-organism infections. One study demonstrated an approximately 6-log increase in CFU of B. fragilis 8 days after infection when coinfected with an Enterichia coli isolate compared with infection with B. fragilis alone (17). Each combination of aerobic isolates tested in this study was tested with and without the presence of B. fragilis. While there was a numeric trend toward smaller reductions of Enterobacteriaceae isolate levels in infections that included the presence of B. fragilis, this did not seem to be substantial, as the average difference with or without the presence of B. fragilis was 0.35 ± 0.23 log₁₀ CFU. These findings suggest that ceftaroline-avibactam is effective in reducing bacterial CFU of concomitant organisms with and without the presence of B. fragilis.

Recent in vitro surveillance data showed that the ceftaroline-avibactam MIC₅₀ and MIC₉₀ of Enterobacteriaceae, S. aureus, and B. fragilis isolates were 0.06 and 0.25, 0.5 and 1, and 0.25 and 2 μg/ml, respectively (7, 18). Another in vitro study with isolates cultured only from diabetic foot wounds showed similar results, with MIC₅₀ and MIC₉₀ values of 0.03 and 0.125, 0.5 and 2 μg/ml for Enterobacteriaceae, MRSA, and B. fragilis isolates, respectively (8). Additionally, the presence of avibactam results in a broader coverage of potential pathogens often involved in polymicrobial infections, such as anaerobes, than that of cefaroline alone.

Given these recent data, the MICs of the organisms used in the current study are within the distributions likely to be observed in patients hospitalized with these infections. Although the current incidence of ESBL-producing Enterobacteriaceae in skin and soft-tissue infections is relatively low in the United States compared to other regions of the world, the numbers of these cases are likely to increase over the next few decades (11).

Murine thigh infection models are often used to represent efficacy in skin and soft-tissue infections. While ceftaroline fosamil-avibactam was found to be efficacious against the polymicrobial infections tested here, the concentrations of this agent at the site of infection in patients has yet to be determined. Understanding the concentrations at the site of infection is especially beneficial in ensuring that adequate pharmacodynamic targets are achieved. Additional research to assess tissue penetration and wound concentrations of ceftaroline fosamil-avibactam would be valuable to further support its role in therapy.

The simulated human dosing of 600 mg ceftaroline fosamil–600 mg avibactam every 8 h as a 1-h infusion showed predictable efficacy against all infections tested in this polymicrobial tissue infection model. Despite the burden of multiple infecting organisms, efficacy against each was observed. Given these in vivo findings and the expanded antimicrobial coverage, including ESBLs and anaerobic organisms, ceftaroline fosamil-avibactam is an attractive monotherapy option for polymicrobial infections, such as those occurring in the diabetic population with lower limb infections.

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REFERENCES


